L6 ANSWER 1 OF 1 USPATFULL
AN 97:114931 USPATFULL
TI Modified anti-ICAM-1 antibodies and their use in the treatment of inflammation
IN Faanes, Ronald Bertrand, Pound Ridge, NY, United States
McGoff, Paul Edward, Watertown, CT, United States
Shirley, Bret Allen, New Milford, CT, United States
Scher, David Stuart, Danbury, CT, United States
PA Boehringer Inglehiem Pharmaceuticals, Inc., Ridgefield, CT, United States (U.S. corporation)
PI US 5695760 19971209
AI US 1995-427355 19950424 (8)
DT Utility
LN.CNT 3085
INCL INCLM: 424/178.100
INCLS: 424/181.100; 530/391.100; 530/388.850
NCL NCLM: 424/178.100
NCLS: 424/181.100; 530/388.850; 530/391.100
IC [6]
ICM: A61K039-395
ICS: C07K016-28
EXF 530/391.1; 530/388.85; 424/181.1; 424/178.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l6 ab kwic

L6 ANSWER 1 OF 1 USPATFULL
AB Methods for preventing or treating inflammation are provided. Specifically, such inflammation can be effectively treated or prevented through the use of anti-ICAM-1 antibodies which have been modified to contain poly(ethylene) glycol adducts. The modification reduces the immunoreactivity of the antibodies, and thus increases the antibodies' serum half life. Methods for forming, purifying and using such modified antibodies are described.
IN **Shirley, Bret Allen**, New Milford, CT, United States
DETD . . . albumin, gelatin, or immunoglobulins; hydrophilic polymers such
as polyvinyl pyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or **arginine**; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose,
mannose, or dextrins; chelating agents such as EDTA; and. . .

=> s l6 and igf

L7 0 L6 AND IGF

=> d his

(FILE 'HOME' ENTERED AT 15:05:02 ON 13 JAN 2001)

FILE 'BIOSIS, MEDLINE, USPATFULL, SCISEARCH' ENTERED AT 15:06:14 ON 13 JAN 2001

```
                E SHIRLEY BERT A/AU
L1            1 S E8
L2            4 S E9
L3            1 S E10
L4            2 S (L1 OR L2 OR L3)  AND (IGF )
L5            0 S L4 AND ARGININE
L6            1 S (L1 OR L2 OR L3) AND ARGININE
L7            0 S L6 AND IGF

=> d l4 1-2


L4   ANSWER 1 OF 2  BIOSIS  COPYRIGHT 2001 BIOSIS
AN   1998:422060  BIOSIS
DN   PREV199800422060
TI   Issues in liquid formulation development for insulin-like growth factor I
     (IGF-I.
AU   Shirley, Bret A.; Bajwa, Kamaljit K.; Lone, Timothy A.;
     Arellano, Sandra L.; Hora, Maninder S.
CS   Dep. Formulation, Chiron Corp., Emeryville, CA 94521 USA
SO   Abstracts of Papers American Chemical Society, (1998) Vol. 216, No. 1-3,
     pp. BIOT 7.
     Meeting Info.: 216th National Meeting of the American Chemical Society
     Boston, Massachusetts, USA August 23-27, 1998 American Chemical Society
     . ISSN: 0065-7727.
DT   Conference
LA   English

L4   ANSWER 2 OF 2  BIOSIS  COPYRIGHT 2001 BIOSIS
AN   1998:105333  BIOSIS
DN   PREV199800105333
TI   A sustained-release system for efficient encapsulation with high loading
     of insulin-like growth factor-I (IGF-I.
AU   Qiang, Ye (1); Stevenson, Mark (1); Asherman, John (1); Chen, Sharon;
     Shirley, Bret; Katre, Nandini (1)
CS   (1) Dep. Tech. Corp., San Diego, CA 92121 USA
SO   Pharmaceutical Research (New York), (Nov., 1997) Vol. 14, No. 11 SUPPL.,
     pp. S469.
     Meeting Info.: Annual Meeting of the American Association of
     Pharmaceutical Scientists Boston, Massachusetts, USA November 2-6, 1997
     American Association of Pharmaceutical Scientists
     . ISSN: 0724-8741.
DT   Conference
LA   English


=> d l6


L6   ANSWER 1 OF 1  USPATFULL
AN     97:114931  USPATFULL
TI     Modified anti-ICAM-1 antibodies and their use in the treatment of
       inflammation
IN     Faanes, Ronald Bertrand, Pound Ridge, NY, United States
       McGoff, Paul Edward, Watertown, CT, United States
       Shirley, Bret Allen, New Milford, CT, United States
       Scher, David Stuart, Danbury, CT, United States
PA     Boehringer Inglehiem Pharmaceuticals, Inc., Ridgefield, CT, United
       States (U.S. corporation)
PI     US 5695760  19971209
AI     US 1995-427355  19950424 (8)
DT     Utility
LN.CNT 3085
INCL   INCLM: 424/178.100
       INCLS: 424/181.100; 530/391.100; 530/388.850
NCL    NCLM:  424/178.100
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NCLS: 424/181.100; 530/388.850; 530/391.100
IC [6]
ICM: A61K039-395
ICS: C07K016-28
EXF 530/391.1; 530/388.85; 424/181.1; 424/178.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 15:05:02 ON 13 JAN 2001)

FILE 'BIOSIS, MEDLINE, USPATFULL, SCISEARCH' ENTERED AT 15:06:14 ON 13 JAN 2001

E SHIRLEY BERT A/AU

L1	1	S	E8
L2	4	S	E9
L3	1	S	E10
L4	2	S	(L1 OR L2 OR L3) AND (IGF)
L5	0	S	L4 AND ARGININE
L6	1	S	(L1 OR L2 OR L3) AND ARGININE
L7	0	S	L6 AND IGF

514/012.000; 514/021.000
IC [6]
ICM: A61K038-21
ICS: A61K038-28; A61K038-29
EXF 424/85.7; 424/85.1; 424/85.2; 424/85.4; 514/3; 514/4; 514/12; 514/21
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 9 OF 13 USPATFULL
AN 97:35684 USPATFULL
TI Reduction of skin irritation and resistance during electrotransport
IN Cormier, Michel J. N., Mountain View, CA, United States
Ledger, Philip W., Bedford, United Kingdom
Johnson, Juanita, Brisbane, CA, United States
Phipps, Joseph B., Maple Grove, MN, United States
Chao, Stella, San Carlos, CA, United States
PA ALZA Corporation, Palo Alto, CA, United States (U.S. corporation)
PI US 5624415 19970429
AI US 1995-427336 19950424 (8)
DT Utility
LN.CNT 1625
INCL INCLM: 604/290.000
INCLS: 604/020.000
NCL NCLM: 604/290.000
NCLS: 604/020.000
IC [6]
ICM: A61N001-30
EXF 604/890.1; 604/20-21; 604/290; 604/49; 604/50; 604/65-66; 607/149-153; 128/783; 128/632

L15 ANSWER 10 OF 13 USPATFULL
AN 96:60160 USPATFULL
TI Reduction of skin irritation during electrotransport
IN Phipps, Joseph B., Plymouth, MN, United States
PA Alza Corporation, Palo Alto, CA, United States (U.S. corporation)
PI US 5533971 19960709
AI US 1993-116660 19930903 (8)
DT Utility
LN.CNT 1612
INCL INCLM: 604/020.000
INCLS: 604/890.100; 604/290.000; 607/115.000
NCL NCLM: 604/020.000
NCLS: 604/290.000; 604/890.100; 607/115.000
IC [6]
ICM: A61N001-30
EXF 604/20; 604/65-66; 604/290; 604/890.1; 607/149-152; 607/115; 436/74; 436/79; 436/55; 128/637

L15 ANSWER 11 OF 13 USPATFULL
AN 96:3504 USPATFULL
TI Transmucosal therapeutic composition
IN Igari, Yasutaka, Kobe, Japan
Yamada, Minoru, Kawanishi, Japan
Taketomi, Shigehisa, Ikeda, Japan
PA Takeda Chemical Industries, Ltd., Osaka, Japan (non-U.S. corporation)
PI US 5482706 19960109
AI US 1993-49402 19930416 (8)
PRAI JP 1992-97947 19920417
DT Utility
LN.CNT 1309
INCL INCLM: 424/085.700
INCLS: 424/085.100; 424/085.200; 424/085.400; 514/003.000; 514/004.000; 514/012.000; 514/021.000
NCL NCLM: 424/085.700
NCLS: 424/085.100; 424/085.200; 424/085.400; 514/003.000; 514/004.000; 514/012.000; 514/021.000
IC [6]

ICM: A61K038-21
ICS: A61K038-28; A61K038-29
EXF 514/3; 514/4; 514/12; 514/21; 424/85.1; 424/85.2; 424/85.4; 424/85.7
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 12 OF 13 USPATFULL
AN 94:40056 USPATFULL
TI Benzo-fused lactams that promote the release of growth hormone
IN Fisher, Michael H., Ringoes, NJ, United States
Schoen, William R., Edison, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
DeVita, Robert J., Westfield, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5310737 19940510
AI US 1993-12190 19930202 (8)
RLI Division of Ser. No. US 1992-839742, filed on 28 Feb 1992, now patented,
Pat. No. US 5206235 which is a continuation-in-part of Ser. No. US 1991-673695, filed on 20 Mar 1991, now abandoned
DT Utility
LN.CNT 6705
INCL INCLM: 514/215.000
INCLS: 540/491.000
NCL NCLM: 514/215.000
NCLS: 540/491.000
IC [5]
ICM: A61K031-55
ICS: C07D281-02
EXF 540/491; 514/213; 514/211; 514/215
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 13 OF 13 USPATFULL
AN 93:33488 USPATFULL
TI Benzo-fused lactams that promote the release of growth hormone
IN Fisher, Michael H., Ringoes, NJ, United States
Schoen, William R., Edison, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
DeVita, Robert J., Westfield, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5206235 19930427
AI US 1992-839742 19920228 (7)
RLI Continuation-in-part of Ser. No. US 1991-673695, filed on 20 Mar 1991, now abandoned
DT Utility
LN.CNT 6831
INCL INCLM: 514/213.000
INCLS: 540/455.000; 540/460.000; 540/461.000; 540/467.000; 540/480.000; 540/491.000; 540/509.000; 540/523.000; 544/052.000; 544/105.000; 544/354.000; 546/157.000; 546/158.000; 548/253.000; 548/486.000
NCL NCLM: 514/210.210
NCLS: 514/211.060; 514/212.070; 514/217.050; 514/217.070; 514/217.080; 540/455.000; 540/460.000; 540/461.000; 540/467.000; 540/480.000; 540/491.000; 540/509.000; 540/523.000; 544/052.000; 544/105.000; 544/354.000; 546/157.000; 546/158.000; 548/253.000; 548/486.000
IC [5]
ICM: A61K031-55
ICS: C07D223-16; C07D285-36; C07D267-14
EXF 546/467; 546/480; 546/523; 546/544; 514/211; 514/213
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l15 1-13

L15 ANSWER 1 OF 13 USPATFULL
AN 2000:131805 USPATFULL
TI Conjugates useful in the treatment of prostate cancer
IN Brady, Stephen F., Philadelphia, PA, United States
Garsky, Victor M., Blue Bell, PA, United States
Pawluczyk, Joseph M., Plymouth Meeting, PA, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 6127333 20001003
AI US 1998-112656 19980709 (9)
PRAI US 1997-52195 19970710 (60)
DT Utility
LN.CNT 2013
INCL INCLM: 514/002.000
INCLS: 514/016.000; 514/017.000; 514/018.000; 530/329.000; 530/330.000; 540/478.000
NCL NCLM: 514/002.000
NCLS: 514/016.000; 514/017.000; 514/018.000; 530/329.000; 530/330.000; 540/478.000
IC [7]
ICM: A61K038-00
EXF 530/329; 530/330; 514/2; 514/16; 514/17; 514/18; 540/478
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 2 OF 13 USPATFULL
AN 2000:64696 USPATFULL
TI Method for removing N-terminal methionine
IN Nishimura, Osamu, Hyogo, Japan
Suenaga, Masato, Hyogo, Japan
Ohmae, Hiroaki, Nara, Japan
Tsuji, Shinji, Hyogo, Japan
PA Takeda Chemical Industries, Ltd., Osaka, Japan (non-U.S. corporation)
PI US 6066470 20000523
AI US 1997-872417 19970610 (8)
PRAI JP 1996-154634 19960614
DT Utility
LN.CNT 2980
INCL INCLM: 435/069.100
INCLS: 435/069.400; 435/069.520; 530/300.000; 530/311.000; 530/331.000; 530/343.000; 530/350.000; 530/351.000; 530/399.000
NCL NCLM: 435/069.100
NCLS: 435/069.400; 435/069.520; 530/300.000; 530/311.000; 530/331.000; 530/343.000; 530/350.000; 530/351.000; 530/399.000
IC [7]
ICM: C12P021-06
ICS: C07K001-107; C07K001-12
EXF 435/69.1; 435/69.4; 435/69.52; 530/300; 530/311; 530/331; 530/343; 530/350; 530/351; 530/399
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 3 OF 13 USPATFULL
AN 2000:12602 USPATFULL
TI S-adenosyl methionine regulation of metabolic pathways and its use in diagnosis and therapy
IN Schwartz, Dennis E., Redmond, WA, United States
Vermeulen, Nicolaas M. J., Woodinville, WA, United States

O'Day, Christine L., Mountlake Terrace, WA, United States
PA Oridigm Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6020139 20000201
AI US 1995-428963 19950425 (8)
DT Utility
LN.CNT 4367
INCL INCLM: 435/007.100
INCLS: 435/007.100; 435/192.000; 514/556.000
NCL NCLM: 435/007.100
NCLS: 435/192.000; 514/556.000
IC [6]
ICM: G01N033-53
ICS: C12N009-08; A01N037-30
EXF 435/7.1; 435/192; 514/556
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 4 OF 13 USPATFULL
AN 2000:4808 USPATFULL
TI Indolocarbazole derivatives useful for the treatment of neurodegenerative diseases and cancer
IN Roder, Hanno, Ratingen, Germany, Federal Republic of
Lowinger, Timothy B., Nishinomiya, Japan
Brittelli, David R., Branford, CT, United States
VanZandt, Michael C., Guilford, CT, United States
PA Bayer Corporation, Pittsburgh, PA, United States (U.S. corporation)
PI US 6013646 20000111
AI US 1998-109131 19980702 (9)
DT Utility
LN.CNT 1457
INCL INCLM: 514/219.000
INCLS: 540/556.000
NCL NCLM: 514/219.000
NCLS: 540/556.000
IC [6]
ICM: A61K031-55
ICS: C07D487-00; C07D491-00
EXF 540/556; 514/219
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 5 OF 13 USPATFULL
AN 1999:92802 USPATFULL
TI Dipeptides which promote release of growth hormone
IN Carpino, Philip A., Groton, CT, United States
Dasilva-Jardine, Paul A., Providence, RI, United States
Lefker, Bruce A., Gales Ferry, CT, United States
Ragan, John A., Gales Ferry, CT, United States
PA Pfizer Inc, New York, NY, United States (U.S. corporation)
PI US 5936089 19990810
WO 9638471 19961205
AI US 1997-973268 19971126 (8)
WO 1995-IB410 19950529
19971126 PCT 371 date
19971126 PCT 102(e) date
DT Utility
LN.CNT 5362
INCL INCLM: 546/143.000
INCLS: 546/146.000; 514/307.000; 514/310.000
NCL NCLM: 546/143.000
NCLS: 546/146.000
IC [6]
ICM: A61K031-475
ICS: C07D217-06
EXF 546/143; 546/146; 514/310
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 6 OF 13 USPATFULL

AN 1999:65248 USPATFULL
TI Osteogenic promoting pharmaceutical composition
IN Hoshino, Tetsuo, Osaka, Japan
Muranishi, Hiroya, Kyoto, Japan
Taketomi, Shigehisa, Osaka, Japan
Iwasa, Susumu, Kyoto, Japan
PA Takeda Chemical Industries, Ltd., Osaka, Japan (non-U.S. corporation)
PI US 5910492 19990608
AI US 1996-719467 19960925 (8)
PRAI JP 1995-138036 19950605
JP 1996-11686 19960126
WO 1996-JP1506 19960604
DT Utility
LN.CNT 1509
INCL INCLM: 514/114.000
INCLS: 514/096.000; 514/119.000; 514/140.000
NCL NCLM: 514/114.000
NCLS: 514/096.000; 514/119.000; 514/140.000
IC [6]
ICM: A61K031-67
ICS: A61K031-66
EXF 514/96; 514/114; 514/119; 514/140
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 7 OF 13 USPATFULL
AN 1998:31024 USPATFULL
TI Bridged piperidines promote release of growth hormone
IN Lu, Zhijian, Scotch Plains, NJ, United States
Patchett, Arthur A., Westfield, NJ, United States
Tata, James R., Westfield, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5731317 19980324
AI US 1995-401849 19950310 (8)
DT Utility
LN.CNT 1591
INCL INCLM: 514/289.000
INCLS: 514/284.000; 546/071.000; 546/072.000; 546/073.000; 546/074.000
NCL NCLM: 514/289.000
NCLS: 514/284.000; 546/071.000; 546/072.000; 546/073.000; 546/074.000
IC [6]
ICM: A61K031-445
ICS: A61K031-46
EXF 546/125; 546/129; 546/130; 546/131; 546/132; 546/1; 546/26; 546/61; 546/71; 546/72; 546/73; 546/74; 546/77; 514/289; 514/284; 514/279; 514/278; 514/277; 514/294
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L15 ANSWER 8 OF 13 USPATFULL
AN 1998:24916 USPATFULL
TI Transmucosal therapeutic composition
IN Igari, Yasutaka, Kobe, Japan
Yamada, Minoru, Kawanishi, Japan
Taketomi, Shigehisa, Ikeda, Japan
PA Takeda Chemical Industries, Ld., Osaka, Japan (non-U.S. corporation)
PI US 5725852 19980310
AI US 1995-526987 19950912 (8)
RLI Division of Ser. No. US 1993-49402, filed on 16 Apr 1993, now patented, Pat. No. US 5482706
PRAI JP 1992-97947 19920417
DT Utility
LN.CNT 1251
INCL INCLM: 424/085.700
INCLS: 424/085.100; 424/085.200; 424/085.400; 514/003.000; 514/004.000; 514/012.000; 514/021.000
NCL NCLM: 424/085.700
NCLS: 424/085.100; 424/085.200; 424/085.400; 514/003.000; 514/004.000;

L17 ANSWER 1 OF 1 USPATFULL
AN 2000:64696 USPATFULL
TI Method for removing N-terminal methionine
IN Nishimura, Osamu, Hyogo, Japan
Suenaga, Masato, Hyogo, Japan
Ohmae, Hiroaki, Nara, Japan
Tsuji, Shinji, Hyogo, Japan
PA Takeda Chemical Industries, Ltd., Osaka, Japan (non-U.S. corporation)
PI US 6066470 20000523
AI US 1997-872417 19970610 (8)
PRAI JP 1996-154634 19960614
DT Utility
LN.CNT 2980
INCL INCLM: 435/069.100
INCLS: 435/069.400; 435/069.520; 530/300.000; 530/311.000; 530/331.000; 530/343.000; 530/350.000; 530/351.000; 530/399.000
NCL NCLM: 435/069.100
NCLS: 435/069.400; 435/069.520; 530/300.000; 530/311.000; 530/331.000; 530/343.000; 530/350.000; 530/351.000; 530/399.000
IC [7]
ICM: C12P021-06
ICS: C07K001-107; C07K001-12
EXF 435/69.1; 435/69.4; 435/69.52; 530/300; 530/311; 530/331; 530/343; 530/350; 530/351; 530/399
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

FILE 'BIOSIS, MEDLINE, USPATFULL, SCISEARCH' ENTERED AT 15:06:14 ON 13 JAN 2001

		E	SHIRLEY BERT A/AU
L1	1	S	E8
L2	4	S	E9
L3	1	S	E10
L4	2	S	(L1 OR L2 OR L3) AND (IGF)
L5	0	S	L4 AND ARGININE
L6	1	S	(L1 OR L2 OR L3) AND ARGININE
L7	0	S	L6 AND IGF
L8	69549	S	IGF OR (INSULIN LIKE GROWTH FACTOR)
L9	1631	S	L8 AND ARGININE
L10	0	S	L9 AND (GLUTARIC ACID BUFFER)
L11	0	S	L9 AND (MATEIC ACID)
L12	0	S	L9 AND (SUCCINIC ACID BUFFER)
L13	84	S	L9 AND CITRIC AND BUFFER
L14	0	S	L13 AND (HISTADINE BUFFER)
L15	13	S	L13 AND IMIDAZOLE
L16	0	S	L15 AND QUANIDINE
L17	1	S	L15 AND GUANIDINE

in **buffer** (50 mM TRIS, 140 mM NaCl, pH 7.2). Assay reactions are run for 0, 30, 60, 120, and 180 minutes. . .

L15 ANSWER 2 OF 13 USPATFULL

DETD . . . oxytocin, proinsulin C-peptide, secretin, somatostatin, thyroid-stimulating hormone releasing hormone, ubiquitin, urogastrone, vasopressin derivatives, kinin derivatives, tuftsin, somatomedin, corticotropin releasing factor, **insulin-like growth factor**, calcitonin gene related peptide, PTHrP, VIP, DHI, insulinotropin, GRP, CCK-PZ, Galanin, Antrum Peptide, motilin, PPY, Pancreatic Polypeptide, PSP, pancreastatin, hCG,. . .

DETD . . . 2,000 to 4,000 moles) of the .alpha.-diketone derivative relative to one mole of the peptide or a salt thereof. Any **buffer** solution (e.g. phosphate **buffer**, acetate **buffer**, citrate **buffer**, etc.) may be used for the above described transamination reaction, as long as it does not inhibit the reaction. Among others, acetate **buffer** is preferably used. The reaction pH is preferably adjusted in the range of about 2 to 9, more preferably about. . .

DETD . . . as sulfonic acid, nitric acid, hydrochloric acid, perchloric acid, etc. or an organic acid such as acetic acid, oxalic acid, **citric** acid, carbonic acid, etc. Among others, copper sulfate and copper acetate are preferably used and, in particular, cupric sulfate is. . .

DETD . . . including an alkylamine derivative such as triethylamine, tributylamine, etc., an aromatic amine derivative such as N,N-dimethylaniline, pyridine, lutidine, collidine, 4-(dimethylamino)pyridine, **imidazole**, etc., and urea can be used. Among others, an aromatic amine derivative is preferable, in particular, pyridine is more preferably. . .

DETD . . . amine including cysteamine, an alkylamine such as triethylamine, tributylamine, etc., an aromatic amine such as N,N-dimethyl-aniline, pyridine, lutidine, collidine, 4-(dimethyl-amino)pyridine, **imidazole**, etc., a diamine such as o-phenylenediamine, tolylene-3,4-diamine, 3,4-diaminobenzoic acid, 2,3-diaminophenol, 4-chloro-o-phenylenediamine (preferably an aromatic diamine, more preferably an o-phenylenediamine), etc.,. . .

DETD . . . 0 to 70.degree. C. (preferably from about 20 to 40.degree. C.).

The reaction is preferably carried out with using a **buffer** solution as a solvent. The **buffer** solution is exemplified by phosphate **buffer** solution, acetate **buffer** solution, citrate **buffer** solution, etc. Any **buffer** solution may be used for the above described transamination reaction, as long as it does not inhibit the reaction. Among others, acetate **buffer** is preferably used. Reaction pH is preferably adjusted in the rage of about 2 to 9, more preferably about 3. . .

DETD Arg: **Arginine**

DETD To 2 kg of wet cells obtained in Reference Example 1 was added 6 L of 50 mM Tris-HCl **buffer** (pH 8.0) containing 8 M guanidine hydrochloride. The mixtures were stirred to dissolve the cells in the **buffer** solution, followed by centrifugation (10,000 rpm, 60 minutes) to obtain 6 L of cell extract solution. Thus obtained solution was. . . the column. The fraction of Met-rhGH was collected by eluting with a linear concentration gradient consisting of 10 mM Tris-HCl **buffer** solution (pH 7.0) containing 40% saturated ammonium sulfate and 10 mM Tris-HCl **buffer** solution (pH 7.0) and was dialyzed against 50 mM sodium hydrogen carbonate (pH 8.2), followed by centrifugation (4,200 rpm, 45. . . The fraction of Met-rhGH was collected by eluting with a linear concentration gradient consisting of 10 mM sodium hydrogen carbonate **buffer** solution (pH 8.2) and 10 mM sodium hydrogen carbonate **buffer** solution (pH 8.2) containing 1 M sodium chloride and was dialyzed against 50 mM sodium hydrogen carbonate (pH 8.2). About. . . Tosoh), followed by

adsorption and eluting with HPLC using a linear concentration gradient consisting of 10 mM sodium hydrogen carbonate **buffer** solution (pH 8.2) and 10 mM sodium hydrogen carbonate **buffer** solution (pH 8.2) containing 1 M sodium chloride and was dialyzed against 50 mM sodium hydrogen carbonate (pH 8.2), to. . .

DETD . . . having methionine at its N-terminal (Met-rhGH) obtained in Reference Example 2 was dissolved in 40 ml of 50 mM phosphate **buffer** solution (pH 8.0). To the mixture was added a solution containing 0.1 M copper sulfate 5 ml, glyoxylic acid 2.3. . . 1 hour.

The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2M acetate-2M sodium acetate **buffer** solution and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-rhGH. To this fraction was. .

for 20 hours. The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM Tris-HCl **buffer** solution (pH 8.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of human growth hormone having no methionine at its N-terminal (rhGH). The collected fraction was loaded on DEAE-5PW column (21.5 mmID.times.150 mmL) equilibrated with 20 mM Tris-HCl **buffer** solution (pH 8.0), followed by eluting with a linear concentration gradient of 0-100% solution B (B=20 mM Tris-HCl **buffer** solution +1 M sodium chloride, pH 8.0) at 8.5 ml/minute of flow rate for

30 minutes to collect the fraction. . .

DETD The powder of rhGH obtained in Working Example 1 was suspended in Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] and the mixture was heated with 100 mM DTT at 100.degree. C. for 1 minutes,. . .

DETD . . . (DTT) and the mixture was homogenized, followed by centrifugation. The resultant pellet was dissolved in 120 ml of 20 mM **citric** acid/8M urea (pH 3.0), followed by centrifugation to separate supernatant and precipitate. The precipitate was treated in the

same manner. . . urea was removed. This solution was allowed to stand

at 4.degree. C. for 2 days, to which 50 mM phosphate **buffer** /12.5% sucrose (pH 6.8) to obtain 8.5 L solution. The solution was adjusted to pH 6.0 using 5M sodium hydroxide or. . .

DETD . . . allowed to pass through SP-Sepharose Fast Flow column (2.5 cm .phi..times.12 cm, Pharmacia Biotech Inc.) equilibrated with 100 mM phosphate **buffer**/0.1% 3-[(3-colamidepropyl)dimethylammonio]-1-propane sulfonate (CHAPS) (pH 6.0), followed by adsorption and washing with 100 mM phosphate **buffer**/0.1% CHAPS/200 mM sodium chloride (pH 6.0). The column was eluted with 100 mM phosphate **buffer** /0.1% CHAPS/400 mM sodium chloride (pH 6.0) to obtain solution containing Met-NT-3. The solution was boated on Resource 15 RPC column. . .

DETD . . . for 15 minutes. The reaction solution was passed through Sephadex G-25 column (2.5 cm .phi..times.59 cm) equilibrated with 2M acetate **buffer** solution (pH 4.9) to collect 36 ml of diketone derivative of Met-NT-3. To this solution was added 78 mg o-phenylenediamine,. . . for 15 hours. The reaction solution was passed through Sephadex G-25 column (2.5 cm .phi..times.59 cm) equilibrated with 2M acetate **buffer** solution (pH 4.9) to obtain the fraction of 76 ml NT-3. The obtained fraction was loaded on ODP-50 column, followed. . .

DETD The powder of NT-3 obtained in Working Example 4 was suspended in Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] and the mixture was heated with 100 mM DTT at 100.degree. C. for 1 minute,. . .

DETD . . . the cultivation was continued for 4 hours. The bacterial cells were collected by centrifugation, and suspended in 0.5 ml of

buffer containing 20 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.5M NaCl, 10% sucrose and 0.25 mM PMSF and then to. . .

DETD The stored cells in the amount of 5 liters were thawed and it was suspended in a 300 ml of **buffer** containing 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.2 M NaCl, 10% sucrose and 1 mM APMSF. To the. . . 10 minutes at 4.degree. C. A precipitate was collected by centrifugation for 20 minutes at 20000.times.g, and suspended in a **buffer** containing 100 ml of 20 mM Tris (pH 7.4), 1 mM EDTA and 1 mM APMSF, and to the resultant. . . hour, and the resulting supernatant was applied to S-Sepharose column (diameter 1.6.times.10 cm: Pharmacia). After washing a column with a **buffer** containing 0.1M potassium phosphate (pH 6), 1 mM EDTA and 1 mM APMSF, a gradient elution was carried out with. . .

DETD . . . having methionine at its N-terminal (Met-BTC) obtained in Reference Example 4(3) was dissolved in 16 ml of 50 mM phosphate **buffer** solution (pH 8.0). To the mixture was added a solution containing 0.1M copper sulfate 2 ml, glyoxylic acid 0.92 g. . . 1 hour. The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2M acetate-2M sodium acetate **buffer** solution and the column washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . . for 20 hours. The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM Tris **buffer** solution (pH 8.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of human betacellulin having no methionine at its N-terminal. . . collected fraction was adjusted to pH 6.0 and loaded on SP-Sepharose column (10 mmID.times.200 mmL) equilibrated with 100 mM phosphate **buffer** solution (pH 6.0), followed by eluting with a linear concentration gradient of 0-100% solution B (B=100 mM phosphate **buffer** solution +1M sodium chloride, pH 6.0) at 2.0 ml/minute of flow rate for 60 minutes to collect the fraction of. . .

DETD The powder of BTC 1 .mu.g obtained in Working Example 6 was suspended in Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] and the mixture was heated with 100 mM DTT at 100.degree. C. for 1 minute,. . .

DETD The resulting supernatant, after dialyzation against a 0.01M Tris-HCl **buffer** solution (pH 8.5), was centrifuged at 19,000.times.g for 10 minutes, again yielding a supernatant. The resulting supernatant was passed through a 50 ml column packed with DE52 (DEAE cellulose, Wattman, UK) equilibrated with a 0.01M Tris-HCl **buffer** (pH 8.5) to adsorb protein; IL-2 was then eluted using a NaCl concentration linear gradient (0.15M NaCl, 1 l), yielding. . .

DETD . . . a column (500 ml capacity) packed with Sephacryl S-200 (Pharmacia, Sweden) previously equilibrated with a 0.1M Tris-HCl (pH 8.0)-1M NaCl **buffer**. Forty milliliters of the resulting active fraction was concentrated to 3 ml using a YM-5 membrane. The resulting concentrate was. . .

DETD 0.5 ml of a 0.005M ammonium acetate **buffer** containing the mixture obtained above (pH 5.0, protein concentration 1.03 mg/ml) was adsorbed on an SP-5PW column for HPLC (0.75.times.7.5 cm, Tosoh) equilibrated with a 0.025M phosphate **buffer** solution (pH 7.4) to elute protein. Column temperature and flow rate were maintained at 35.degree. C. and 0.5 ml/min. respectively.. . . .

DETD . . . of methionyl human interleukin-2 (Met-IL-2) obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution containing 25 mM copper sulfate 3.375 ml, glyoxylic acid 1.55. . . passed at 300 ml/hour of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone

to derivative of Met-IL-2. To this fraction was added o-phenylenediamine give. . . passed at 300 ml/hour of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of human interleukin-2 (IL-2). The collected IL-2 fraction was loaded on a SP-5PW column equilibrated with 25 mM phosphate **buffer** solution (pH 7.0), followed by eluting with a pH gradient of 30-80% solution B (B=25 mM phosphate, pH 8.0) at. . .

DETD The powder of IL-2 3 .mu.g obtained in Working Example 8 was suspended in Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] and the mixture was heated with 100 mM DTT at 100.degree. C. for 5 minutes,. . .

DETD To the hGH solution obtained in Working Example 10 was added the same volume of Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] containing 100 mM DTT, and the mixture was heated at 95.degree. C. for 2 minutes,. . .

DETD . . . membrane YM 10, 43 mm; Amicon). To the obtained solution 2 ml, was added 2 ml solution containing 1 M **imidazole**, 0.5 M glyoxylic acid, 20 mM copper sulfate and 2.5 M urea and the mixture was stirred and allowed to. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . . for 15 hours. The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 50 mM phosphate **buffer** solution (pH 6.0) and the column washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of BTC having no methionine at its N-terminal. The. . . pH 6.0 and loaded on SP-5PW column (7.5 mmID.times.75 mmL, Tosoh) equilibrated with 200 mM NaCl and 100 mM phosphate **buffer** solution (pH 5.0), followed by eluting with a linear concentration gradient of 0-100% solution B (B=100 mM phosphate **buffer** solution+200 mM NaCl, pH 9.0) at 0.8 ml/minute of flow rate for 30 minutes to collect the fraction of BTC.. . .

DETD To the BTC solution obtained in Working Example 23 was added Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] containing 100 mM DTT, and the mixture was heated at 95.degree. C. for 1 minute,. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the

fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . dissolved in 2 ml of 3 M urea solution. To the mixture was added 2 ml solution containing 1 M **imidazole**, 0.5 M glyoxylic acid, 20 mM copper sulfate and 3 M urea and allowed to stand at 25.degree. C. for. . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-BTC. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. . .

for 15 hours. The reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 50 mM phosphate **buffer** solution (pH 6.0) and the column washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of NT-3 having no methionine at its N-terminal. The. . . pH 6.0 and loaded on SP-5PW column (21.5 mmID.times.150 mmL, Tosoh) equilibrated with 200 mM NaCl and 100 mM phosphate **buffer** solution (pH 5.0), followed by eluting with a linear concentration gradient of 0-100% solution B (B=100 mM phosphate **buffer** solution+200 mM NaCl, pH 9.0) at 6 ml/minute of flow rate for 55 minutes to collect the fraction of NT-3.. . .

DETD To the NT-3 solution obtained in Working Example 37 was added Sample **buffer** [Laemmli, Nature, 227, 680 (1970)] containing 100 mM DTT, and the mixture was heated at 100.degree. C. for 1 minute,. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. . .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the

fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. .

DETD . . . dissolved in 2 ml of 3 M urea solution. To the mixture was added 2 ml solution containing 1 M **imidazole**, 0.5 M glyoxylic acid, 20 mM copper sulfate and 3 M urea and allowed to stand at 25.degree. C. for. . . reaction solution was passed through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 2.5 M urea and 50 mM phosphate **buffer** solution (pH 6.0) and the column was washed with the same **buffer** solution at 6 ml/minute of flow rate to collect the fraction of diketone derivative of Met-NT-3. To this fraction was. . .

DETD . . . 3(5) is centrifuged (10000 rpm) and the obtained supernatant was diluted to about 20 times volume with 100 mM phosphate **buffer** solution (pH 8.5) containing 1.8 M urea, 0.2 M Arg, 0.2 mM GSSG and 1.0 mM GSH. The mixture is. . .

DETD . . . the solution is adjusted to pH 6.0 and loaded on SP-Sepharose column (22 mmID.times.120 mmL) equilibrated with 100 mM phosphate

buffer solution (pH 6.0), followed by eluting with 100 mM phosphate **buffer** solution+400 mM NaCl (pH 6.0) to collect the fraction of Met-NT-3. The collected fraction is passed through ODP-50 column (21.5. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added 3.375 ml solution containing 1.55 g glyoxylic acid, 0.1 M nickel chloride,. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added 3.375 ml solution containing 1.55 g glyoxylic acid, 0.1 M cobalt chloride,. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added 3.375 ml solution containing 1.55 g glyoxylic acid, 0.1 M zinc sulfate,. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added 3.375 ml solution containing 1.55 g glyoxylic acid, 0.1 M copper acetate,. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution containing 1.55 g glyoxylic acid, 0.1 M copper sulfate 3.375. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. This fraction was adjusted to pH 8.5 and. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution containing 1.55 g glyoxylic acid, 0.1 M copper sulfate 3.375. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added tolylene-3,4-diamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution

containing 1.55 g glyoxylic acid, 0.1 M copper sulfate 3.375. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added 4-chloro-o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution containing 1.55 g glyoxylic acid, 0.1 M copper sulfate 3.375. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added 3,4-diaminobenzoic acid to. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added a solution containing 1.55 g glyoxylic acid, 0.1 M copper sulfate 3.375. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added 2,3-diaminophenol to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added the same volume of a solution containing 0.5 M glyoxylic acid, 20 mM zinc sulfate, 40 mM ammonium acetate **buffer** solution (pH 5.0) and 6 M urea and allowed to stand at room temperature for 1 hour. The reaction solution. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added the same volume of a solution containing 0.5 M glyoxylic acid, 20 mM copper acetate, 40 mM ammonium acetate **buffer** solution (pH 5.0) and 6 M urea and allowed to stand at room temperature for 1 hour. The reaction solution. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added the same volume of a solution containing 0.5 M glyoxylic acid, 20 mM nickel chloride, 40 mM ammonium acetate **buffer** solution (pH 5.0) and 6 M urea and allowed to stand at room temperature for 1 hour. The reaction solution. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0). To the mixture was added the same volume of a solution containing 0.5 M glyoxylic acid, 20 mM cobalt chloride, 40 mM ammonium acetate **buffer** solution (pH 5.0) and

6 M urea and allowed to stand at room temperature for 1 hour. The reaction solution. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0), followed by dialysis against 2 l solution of 20 mM Tris-HCl (pH 8.0) containing 3 M urea. To the dialyzed solution was added the same volume of a solution containing 1 M **imidazole**, 0.5 M glyoxylic acid, 20 mM copper sulfate and 2.5 M urea and allowed to stand at room temperature for. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD . . . having methionine at its N-terminal obtained in Reference Example 5 was dissolved in 27 ml of 20 mM ammonium acetate **buffer** solution (pH 5.0), followed by dialysis against 2 l solution of 20 mM Tris-HCl (pH 8.0) containing 3 M urea.. . . passed at 300 ml/h of flow rate through Sephadex G-25 column (25 mmID.times.600 mmL) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of diketone derivative of Met-IL-2. To this fraction was added o-phenylenediamine to give. . .

DETD To 10 g of wet cells obtained in Reference Example 10 was added 20 mM ammonium acetate **buffer** solution 40 ml (pH 5.0) containing 7 M guanidine hydrochloride and the cells were dissolved in the solution , followed. . . about 40 ml of cell extract. The cell extract was diluted with about 2 l of 20 mM ammonium acetate **buffer** solution (pH 5.0), followed by centrifugation (4200 rpm, 20 minutes) to obtain about 2 l of the resultant supernatant. The. . . 650M column (5 cm .phi..times.30 cm), followed by adsorption and washing. The column was eluted with 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 1 M NaCl. The eluted solution was loaded on ODS-120T column (21.5 cm .phi..times.30 cm, Tosoh). . . was loaded on SP-Toyopearl 650M column (1.0 cm .phi..times.30 cm) and the column was washed with 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 100 mM NaCl to remove the reaction agent, followed by elution with 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 1 M NaCl. The eluted solution was dialyzed against 5 l solution of 6 M urea.. . . reaction solution was subjected to gel filtration with Toyopearl HW-50F (2 cm .phi..times.50 cm) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) to collect the fraction of Met-PTH(1-34). The fraction was loaded on ODS-120T column (21.5 cm .phi..times.30 cm,. . .

DETD . . . Showa Denko) using HPLC method, followed by elution with a linear concentration gradient consisting of (1) 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 10% acetonitrile and (2) 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 60% acetonitrile to obtain about 5.4 ml fraction of the diketone derivative of Met-PTH(1-34). The fraction. . . containing 0.1% TFA to obtain the fraction of PTH(1-34). The fraction was diluted 5 times with 20 mM ammonium acetate **buffer** solution (pH 5.0) and the diluted solution was loaded on SP-5PW column (7.5 mm .phi..times.75 mm, Tosoh) equilibrated with 20 mM ammonium acetate **buffer** solution (pH 5.0) containing 2 M urea, followed by elution with a linear concentration gradient consisting of 0-50% solution B. . .

DETD The PTH(1-34) powder obtained in Working Example 66 was suspended in Sample **buffer** [NOVEX JAPAN], and the mixture was subjected to

electrophoresis with Peptide-PAGE mini [TEFCO]. After electrophoresis, the gel was stained with. . .

DETD . . . 3 M urea and 20 mM Tris-HCl (pH 8.0). To the mixture was added 1 ml solution containing 1 M **imidazole**, 0.5 M glyoxylic acid, 20 mM copper sulfate and 2.5 M urea, and the mixture was allowed to stand at. . .

L15 ANSWER 3 OF 13 USPATFULL

DETD . . . histidine derivative diphthamide occurs uniquely in eukaryotic EF-2. The biosynthesis involves transfer of an amincoarboxypropyl moiety from SAM to the **imidazole** ring of histidine to yield 2-(3-amino-3-carboxypropyl)histidine and 5'-deoxy-5'-methylthioadenosine (MTA). MTA is an inhibitor of the diphthamide formation in EF-2. Additionally,. . .

DETD . . . functions as a prosthetic group in carboxylations, transcarboxylations and decarboxylations. Biotin is therefore required for replacement of components of the **citric** acid cycle that have been removed for biosynthetic purposes, including synthesis of oxaloacetate from pyruvate and synthesis of succinyl CoA. . . isoleucine or methionine. Biotin also mediates the reactions which convert leucine to HMG CoA, which in turn can replenish a **citric** acid cycle intermediate (i.e., acetyl CoA) or which can function as precursor to intermediates in the synthesis of isopentenyl derivatives,.

. .

DETD Biotin-deficient cells have blocks in the synthesis of purines and carbamyl phosphate (a direct precursor of **arginine** and the pyrimidines) The enzymatic steps blocked involved CO.sub.2 fixation which implicates biotin as being involved in CO.sub.2 fixing reactions.

. .

DETD . . . is made available to a human in a morning meal containing carbohydrate, energy is produced mainly by glycolysis and the **citric** acid cycle. After several hours, glucose and glycogen stores are nearly depleted and alternative energy stores must be mobilized. Fatty. . . acids, which subsequently are converted to acetyl CoA, pyruvate, or succinyl CoA (all of which are subsequently oxidized in the **citric** acid cycle to produce ATP). As these proteins are degraded, however, free trimethyllysine will be produced and converted to carnitine.. . . acid transport and oxidation can be terminated by degradation of carnitine, and sugars can be oxidized by glycolysis and the **citric** acid cycle to produce the required ATP.

DETD . . . number of growth factors enhance wound healing, including epidermal growth factor (EGF) (Heck et al., J. Biol. Chem. 267:21277-88 (1992)), **insulin-like growth factor** (ILGF) (Olanrewaju et al., Am. J. Physiol. 263:E22-26 (1992)), platelet-derived growth factor (PDGF), transforming growth factor .beta. (TGF.beta.) and basic. . .

DETD SAM, biotin, methionaine, ethylene, polyamines (putrescence, spermine, spermidine), ornithine, **arginine**, 1-aminocyclopropanecarboxylic acid (ACC), queuine, queuosine, calmodulin, fibronectin, protein kinase C, EGF, ILGF, PDGF, TGF.beta. and bFGF.

DETD SAM, biotin, ethylene, 1-aminocyclopropanecarboxylic acid (ACC), polyamines (putrescine, spermine, spermidine), ornithine, **arginine**, methionine , adenosine

DETD calmodulin, fibronectin, protein kinase C epidermal growth factor (EGF), **insulin-like growth factor** (ILGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-beta) and basic fibroblast growth factor (bFGF), nicotinamide, inositol.

DETD SAM, biotin, ethylene, 1-aminocyclopropanecarboxylic acid (ACC), polyamines (putrescine, spermine, spermidine), ornithine, **arginine**, methionine , adenosine, queuine, queuosine, Wye base.

DETD calmodulin, fibroneclin, protein kinase C epidermal growth factor (EGF), **insulin-like growth factor** (ILGF), platelet-derived growth factor (PDGF), transforming growth factor .beta. (TGF-.beta.), basic fibroblast growth factor (bFGF), nicotinamide, inositol.

DETD . . . ice until it becomes liquid. The cell pellet volume is estimated and the cells suspended in 3 volumes of lysis **buffer** (15 mM KCl, 60 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM EDTA, 10% glycerol; to this was added. . . unbound material or flow-through is saved as "Wash A". The affinity matrix is washed 3 times with 0.5 ml loading **buffer** (15 mM KCl, 60 mM NaCl, 50 nM Hepes, pH 7.5, 1 mM EDTA, 1 mM DTT] by gentle mixing for 5 minutes followed by centrifugation for 60 second at 1000.times.g. **Buffer** A (250 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM EDTA, 1 mM DTT] in a volume of 0.5. . . 60 seconds at 1000.times.g. The supernatant from this wash is collected as "Fraction A". This step is repeated successively with **Buffer** B (750 mM NaCl, 50 mM Hepes, pH 7.5, 1 mM EDTA, 1 mM DTT) and **Buffer** C (7M urea, 50 mM Hepes, pH 7.5, 1 mM EDTA, 1 mM DTT) to yield "supernatant B" and "supernatant. . .

DETD . . . generic protocol was designed to perform this analysis. In this approach, three eluting buffers are used: a low ionic strength **buffer** A (0.3M) to elute molecules with relatively low binding affinity; a relatively high ionic strength **buffer** B (0.8M) to elute molecules with relatively high binding affinity and a denaturing **buffer** C (7M Urea) to elute any material with high binding affinity to the ligand on the affinity matrix. This procedure. . .

DETD The methylation states of MBP is critical for myelination. Although MBP has 18 **arginine** residues, it is methylated only at position 107. Two methylation products are produced, N.sup.G -monomethyl **arginine** (NMeArg) and symmetrical N.sup.G,N.sup.G -dimethyl **arginine**. The few other proteins which are methylated at **arginine** residues, such as histones, high-mobility group proteins 1 and 2, A1 protein from HnRNP and the 34 kD nucleolar protein, do not exhibit symmetric dimethylation, and contain only NMeArg and asymmetrical N.sup.G,N'.sup.G -dimethyl **arginine**.

L15 ANSWER 4 OF 13 USPATFULL

DETD . . . not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulfonic, tartaric, **citric**, methane sulfonic, formic, malonic, succinic, naphthalene-2-sulfonic, and benzene sulfonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or. . .

DETD Suitable buffering agents include: acetic acid and a salt (1-2% W/V); **citric** acid and a salt (1-3% W/V); and phosphoric acid and a salt (0.8-2% W/V).

DETD . . . anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; **arginine** deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists;. . . inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; **insulin-like growth factor**-1 receptor inhibitor; interferon

agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F;. . .

DETD . . . minutes, no starting material was observed by TLC (20% EtOAc-CH.sub.2 Cl.sub.2) and the reaction mixture was quenched with a saturated **citric** acid solution (3.0 mL). After warming to room temperature, the solution was diluted with brine (15 mL) and extracted with. . .

DETD . . . from 0.5-1 [cultures were freeze-thawed at -78.degree. C. and homogenized by ultra-sonication for 3 min in 15 ml Ni.sup.2+ -column **buffer** (20 mM Tris HCL pH 7.9, 0.5 M NaCl, 5 mM **imidazole**, Novagen). After centrifugation at 35,000.times. g for 30 min supernatants were loaded onto a 1 ml Ni.sup.2+ charged resin (Novagen). After washing with column **buffer** containing 60 mM **imidazole** the ERK2 protein was eluted with column **buffer** containing 1 M **imidazole**. ERK2 containing fractions were identified by SDS-PAGE and dialyzed into Mono Q A-**buffer** (25 mM Tris HCL, pH 7.5 25 mM NaCl, 1 mM EDTA). The dialysate was loaded onto a HR5/5 Mono Q FPLC column (Pharmacia) and eluted with a 30 ml gradient from Mono Q A-**buffer** to the same **buffer** containing 250 mM NaCl (Mono Q B-**buffer**) at 1 ml/min collecting 30 fractions. Fractions #19-20 and #27-28 typically contained the peak amounts of two ERK2 conformers, as. . . ml gradient from 25 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT to the same **buffer** containing 25 mM NaCl and 60% ethylene glycol with a flow rate from 0.5 ml/min decreasing to 0.1 ml/min at. . .

DETD . . . and incubated for 2 hrs at 37.degree. C. The mixture was dialyzed twice into 11 each of Ni.sup.2+ column binding **buffer** (20 mM Tris, pH 2.9, 500 mM NaCl, 5 mM **imidazole**) to remove traces of DTT and loaded onto 0.3 mL Ni.sup.2+ charged resin (Novagen) at 25 mL/hr. After washing with 10 mL column **buffer** followed by 4 mL column **buffer** containing 40 mM **imidazole** homogeneous activated PK40.sup.erk2 was eluted with 4 mL column **buffer** containing 1 M **imidazole**. The product was dialyzed extensively into 10 mM HEPES, pH 7.0, 1 mM EDTA to remove **imidazole** and traces of Ni.sup.2+, and finally into 10 mM HEPES, pH 7.0, 1 mM EDTA, 1 mM DTT.

DETD . . . sedimented for 12 seconds at 14,000.times. g. The supernatant was removed and cells were lysed in 250 .mu.l cold homogenization **buffer** (50 mM MES, pH 5.8, 5 mM sodium pyrophosphate, 50 mM p-nitrophenylphosphate, 1 .mu.M okadaic acid, 2 mM Na-orthovandate, 1. . .

DETD . . . for 2.5 hors and Western-blotted on nitrocellulose (Novex) overnight at 23 volts or 1.5 hrs at 100 V in transfer **buffer** [Towbin et al. Proc. Natl. Acad. Sci. USA 76, 4350-4354 (1979)] at 4.degree. C. Blots were analyzed for ERK2 and. . .

DETD . . . Hippocampi were cut into 450 mM slices using a McIlwain tissue chopper and placed into ice cold low Ca.sup.2+ Krebs-Bicarbonate **buffer** (pH 7.) of the following composition in mM: NaCl, 124; KCL, 3.33; CaCl.sub.2, 0.01; KH.sub.2 PO.sub.4, 1.25; MgSO.sub.4 1.33; nAhco.sub.3,. . . 25.7; D-glucose, 10; HEPES, 20. The slices were separated and placed, 5-8 per tube, into 5 mL of low Ca.sup.2+ **buffer** and incubated for at least 30 min at 33-34.degree. C. with water saturated oxygenation (95% O.sub.2, 5% CO.sub.2). After 30 min the solution was replaced with **buffer** containing a physiological level of Ca.sup.2+ (1.3 mM) and incubated for an additional 30 min.

DETD . . . 10 .mu.M for 1 hr, and then exposed to either vehicle or okadaic acid fora 90 min. After treatment, the **buffer** was removed and the slices were sonicated for 10-20 sec in 500 .mu.l of homogenization **buffer** (100 mM KH.sub.2 PO.sub.4, pH 6.5, 2 mM EGTA, 2 mM EDTA, 1 .mu.M okadaic acid and the following protease. . .

L15 ANSWER 5 OF 13 USPATFULL

SUMM Various ways are known to release growth hormone. For example, chemicals

such as **arginine**, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth. . . .

SUMM . . . other growth hormone secretagogues such as, GHRP-6, Hexorelan, GHRP-1, growth hormone releasing factor (GRF) or one of its analogs or **IGF**-1 or **IGF**-2, or B-HT920; and

SUMM . . . growth hormone releasing hormone (GHRH, also designated GRF) and its analogs or growth hormone and its analogs or somatomedins including **IGF**-1 and **IGF**-2 or .mu.-adrenergic agonists such as clonidine or serotonin 5HTID agonists such as sumitriptan or agents which inhibit somatostatin or its. . .

DETD . . . by hydrogenation using a catalyst such as palladium in a solvent such as acetic acid with ammonium acetate as a **buffer** and gives compounds of formula 68. ##STR38##

DETD . . . (3.00 mmol) of 4-methanesulfonyloxy-piperidine-1-carboxylic acid tert-butyl ester (Yoon et al., WO9204342) in 3 mL of DMSO was added to the **imidazole** solution, and the mixture was stirred at room temperature for 12 h. The mixture was diluted with ethyl acetate and.

DETD . . . at room temperature. The reaction mixture was diluted with 20 mL of ethyl acetate and washed twice each with 10% **citric** acid and saturated aqueous sodium bicarbonate and once with brine. The solution was dried over MgSO.sub.4 and concentrated to give. . .

DETD . . . mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed twice with 10% **citric** acid, four times with a saturated aqueous sodium bicarbonate and once each with water and brine. The solution was dried. . .

DETD . . . concentration and the remaining aqueous solution was diluted with 200 mL of water and acidified to pH 5 with 10% **citric** acid. The aqueous portion was extracted twice with ethyl acetate. The combined organics were washed twice with saturated aqueous sodium. .

L15 ANSWER 6 OF 13 USPATFULL

SUMM . . . acid, acetic acid, trifluoroacetic acid, oxalic acid, tartaric acid, fumaric acid, maleic acid, methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid and **citric** acid, and such basic or acidic amino acids include **arginine**, lysine, aspartic acid and glutamic acid.

SUMM . . . isopentanoic acid or trichloroacetic acid, or an aromatic carboxylic acid such as benzoic acid; symmetric acid anhydrides; activated amides with **imidazole**, 4-substituted **imidazole**, dimethylpyrazole, triazole or tetrazole; activated esters such as cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl ester, vinyl ester, propargyl ester, p-nitrophenyl ester,. . .

SUMM . . . protein (e.g., gelatin), seaweed (e.g., agar), polysaccharide (e.g., alginic acid), synthetic high-molecular substance (e.g., polyvinyl alcohol), basic amino acid (e.g., **arginine**, lysine) or the like. The internal aqueous phase may be supplemented with an organic acid such as acetic acid, oxalic acid or **citric** acid, an inorganic acid such as carbonic acid or phosphoric acid, an alkali metal hydroxide such as sodium hydroxide, a basic amino acid such as **arginine** or lysine or a salt thereof (e.g., salts with organic acids such as acetic acid, oxalic acid, **citric** acid or salts with inorganic acids such as carbonic acid, phosphoric acid and hydrochloric acid) as a pH regulator for. . . promoting substance, there may be added a protein (e.g., albumin, gelatin), starch derivative (e.g. dextrin, pullulan etc.), organic acid (e.g., **citric** acid), ethylenediaminetetraacetic acid alkali metal salt (e.g., sodium ethylenediaminetetraacetate), sulfurous acid hydrogen alkali metal salt

(e.g., sodium hydrogen sulfite), synthetic. . .
SUMM . . . hyarulonic acid, and polysorbate), a preservative (e.g., methyl
paraben, propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose), **buffer** (e.g. calcium carbonate), pH adjusting agent (e.g. sodium phosphate, potassium phosphate), etc., and may be also prepared as an oily. . .
SUMM . . . fluoride), vitamin K.sub.2, BMP (bone morphogenetic protein), FGF (fibroblast growth factor), PDGF (platelet derived growth factor), TGF-.beta. (transforming growth factor-.beta.), **IGF**-1 (**insulin like growth factor**-1), **IGF**-2 (**insulin like growth factor**-2), PTH (parathyroid hormone), and so on.
DETD . . . C. water bath (TAITEC, incubator M-100, 115 strokes/minute) in the presence of 10 ml of a release test solution (phosphate **buffer** supplemented with 10% bovine serum albumin, pH 7.0). To each 100 .mu.l sample taken over time, 100 .mu.l of acetonitrile. . .

L15 ANSWER 7 OF 13 USPATFULL
SUMM Various ways are known to release growth hormone. For example, chemicals
such as **arginine**, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth. . .
SUMM . . . 5 by reacting 4 with reagent 8, wherein X is an appropriate leaving group such as Cl, Br, I, or **imidazole**. Alternatively, 4 can be reacted with an isocyanate of formula 9 in an inert solvent such as 1,2-dichloroethane which results. . .
SUMM . . . growth hormone releasing hormone (GHRH, also designated GRF) and its analogs or growth hormone and its analogs or somatomedins including **IGF**-1 and **IGF**-2 or .alpha.-adrenergic agonists such as clonidine or serotonin 5HTID agonists such as sumitriptan or agents which inhibit somatostatin or its. . . release such as physostigmine and pyridostigmine. For example, a compound of the
present invention may be used in combination with **IGF**-1 for the treatment or prevention of obesity. In addition, a compound of this invention may be employed in conjunction with. . .
DETD . . . was further extracted with 2.times.100 mL of chloroform. The combined organic solution was washed with 50 mL of 10% aqueous **citric** acid, 100 mL of 10% aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, filtered and concentrated to give a. . .
DETD . . . was further extracted with 2.times.100 mL of chloroform. The combined organic solution were washed with 50 mL of 10% aqueous **citric** acid, 100 mL of 10% aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, filtered and concentrated to give 6.94. . .
DETD . . . with ether (100 mL) and poured into ice-water. The organic layer was separated and the aqueous fraction was acidified with **citric** acid (20%), then extracted with EtOAc. The EtOAc extracts were washed with brine, dried over magnesium sulfate, filtered and evaporated. . .
DETD . . . stirred at 60.degree. C. under nitrogen for 1 hr. The color of the solution changed from yellow to dark brown. **Buffer** solution (pH-7) was added and THF was removed. The aqueous was extracted
with EtOAc (3.times.50 ml). The combined EtOAc layers. . .

L15 ANSWER 8 OF 13 USPATFULL
DETD The growth factors mentioned above include nerve growth factors (NGF, NGF-2/NT-3), epidermal growth factor (EGF), fibroblast growth factor (FGF), **insulin-like growth factor** (**IGF**), transforming growth factor (TGF), platelet-derived cell growth factor (PDGF), hepatocyte growth factor (HGF) and so on.
DETD . . . base necessary for dispersion of such substances. Said

additives include but are not limited to pH control agents, such as **arginine**, sodium hydroxide, glycine, hydrochloric acid, **citric** acid, etc., local anesthetics represented by benzyl alcohol, isotonizing agents such as sodium chloride, mannitol, sorbitol, etc., adsorption inhibitors such. . .

DETD . . . sodium lauryl sulfate, sodium myristyl sulfate, polyoxyethylene alkyl ethers, polyoxyethylene alkyl esters, etc.), caproic acid, lactic acid, malic acid and **citric** acid and alkali metal salts thereof, pyrrolidonecarboxylic acids, alkylpyrrolidonecarboxylic acid esters, N-alkylpyrrolidones, proline acyl esters and so on. While the. . .

DETD . . . form of p-bromobenzyloxycarbonyl ester, the carboxyl group of glutamic acid or aspartic acid in the form of benzyl ester, the **imidazole** nitrogen of hystidine with benzyloxy methyl, the side-chain amino group of lysine with 2-chlorobenzyloxycarbonyl, the guanidino group of **arginine** with p-toluenesulfonyl, and the indoleimine group of tryptophan with formyl. All the amino acids were obtained from Applied Biosystems Japan. . .

DETD In 155.5 .mu.l of 1/10M **citric** acid **buffer** (pH 3.5) was suspended 16.75 mg of human insulin (Wako Pure Chemical Industries). A 20 .mu.l of this suspension was. . .

DETD In 155.5 .mu.l of 1/10M citrate **buffer** (pH 3.5) was dispersed 16.75 mg of human insulin (Wako Pure Chemical) and 50 .mu.l of this dispersion was blended. . .

L15 ANSWER 9 OF 13 USPATFULL

SUMM . . . for example Jacobsen et al U.S. Pat. No. 4,416,274 (sodium phosphate buffers) and Hillman et al U.S. Pat. No. 5,088,978 (**citric** acid/citrate salt buffers). Although conventional buffers are effective to maintain donor reservoir pH, they introduce undesirable extraneous ions which tend. . . anodic donor reservoir for delivering a cationic drug D.sup.+ is buffered with a citrate salt (eg, sodium citrate), the citrate **buffer** absorbs hydronium ions produced by water hydrolysis at the anode but leaves extraneous sodium ions which compete with the drug. . .

SUMM . . . blistering of the skin in contact with either the cathode or anode is dependent upon both the pH and the **buffer** composition of the anodic and cathodic reservoirs.

SUMM . . . Del. Rev. (1992), 9, 239-264, the preferred pH range for avoiding skin irritation for the donor reservoir, independent of the **buffer** used, is 3 to 8. Outside this pH range, according to this reference, irritation and/or damage of the stratum corneum. . .

SUMM In a further preferred embodiment, the pH of one or both of the reservoirs is maintained using a suitable **buffer**. Most preferably, the cathodic reservoir is buffered using a cationic **buffer** and/or the anodic reservoir is buffered using an anionic **buffer**. Most preferably, the cathodic and/or anodic electrodes are composed of electrochemically reactive materials, ie, an electrochemically oxidizable anode and an. . .

DETD . . . cathode) and/or for short periods of time (eg, <1 hour), it may be sufficient to simply add an acid (eg, **citric** acid) to the cathodic reservoir to maintain the desired pH. However, while acids are effective in achieving a low cathodic. . . reservoir pH, they introduce undesirable competing ions in those instances where the cathodic electrode is the donor electrode. Thus, adding **citric** acid to a cathodic donor reservoir containing salicylate anions undesirably adds citrate ions which compete with the salicylate ions for. . .

DETD Preferably, the cathodic reservoir contains at least one cationic

buffer. A **buffer** cation within the cathodic reservoir will tend not to be electrotransported through the skin since anions, and not cations, are predominantly delivered from the cathodic reservoir by electrotransport. A poorly transported **buffer** is preferred in order to avoid depletion of the **buffer** from the reservoir as well as any irritation associated with **buffer** ion being transported into the skin. Amino acids are preferred cationic buffers. Preferably, the counter ion, ie anion, to the **buffer** cation is chloride. In many cases, the counter anions to the **buffer** ions are transported into the skin from the cathodic reservoir. Chloride is a preferred counter anion because the skin has. . .

DETD The concentration of **buffer** required in the reservoir will depend on the properties of the specific **buffer** selected. Generally, the **buffer** concentration will range from about 0.01M to about 1.0M. Preferably, the **buffer** concentration will be about 0.01M to about 0.50M. More preferably, the **buffer** concentration will be about 0.01M to about 0.20M.

DETD . . . Amino Acid Buffers for Cathodic Reservoir

AMINO ACID	pH RANGE FOR CATIONIC BEHAVIOR	PREFERRED pH RANGE FOR CATIONIC BEHAVIOR
histidine	1-5	2-4
lysine	1-4	1.5-3.5
arginine	1-4	1.5-3.5
aspartic acid	1-3	2-3
glutamic acid	1-3.2	2-3.2
cysteine	1-4	2-3
tyrosine	1-4	2-3
other amino acids	1-4	2-3.5

DETD Alternatively, the cathodic reservoir may be buffered using an anionic or negatively charged **buffer**, which is electrotransported through the skin, or alternatively, mixtures of a cationic **buffer** from Table 1 and an anionic **buffer** from Table 2 may also be used. However, the cationic buffers of Table 1 are preferred, particularly when the cathodic electrode is the donor electrode, since **buffer** cations will not be electrotransported through the skin. Thus, irritation from the presence of a **buffer** ion in the skin is minimized, as discussed above. The preferred anionic buffers include those named in Table 2.

DETD TABLE 2

Anionic Acid Buffers for Cathodic Reservoir

BUFFER	pH RANGE FOR ANIONIC BEHAVIOR	PREFERRED pH RANGE FOR ANIONIC BEHAVIOR
aspartic acid	3-5	3-4
glutamic acid	3.2-5	3.2-4
citric acid	1-5	2-4
succinic acid	2-5	3-4
phosphoric acid		

1-5 2-4
acetic acid
3.5-5 3.5-4
EDTA 1-5 2-4
lactic acid
2.7-4.5 2.7-4
benzoic acid
3-5 3-4
tartaric. . .

DETD . . . no buffering capacity may optionally be incorporated into the cathodic reservoir. Such additives may be advantageous in decreasing the potential **buffer** depletion from the reservoir. A disadvantage of adding sodium chloride to the reservoir, at least in the case where the. . .

DETD . . . (eg, platinum or stainless steel), the anodic reservoir is preferably buffered at a pH above about 4. More preferably, the **buffer** has a relatively low anodic electrotransport rate through the skin. Preferred buffers include amino acids exhibiting anionic behavior at a. . .

DETD . . . Reservoir

AMINO ACID	pH RANGE FOR ANIONIC BEHAVIOR	PREFERRED pH RANGE FOR ANIONIC BEHAVIOR
histidine	7.5-10.5	7.5-10
cysteine	7-12	7.5-11.5
tyrosine	7.8-11.4	8.3-10.9
lysine	9.7-11.8	9.7-11.3
arginine	10.8-13	10.8-12
aspartic acid	3-5.2	4-4.6
	8.5-11.1	9.1-10.5
glutamic acid	3.2-5.5	4-5
	8.4-11	8.9-10.4
other amino acids	8-12	9-11

DETD . . . for the anodic reservoir. Examples of such acids can be found in Table 5. The preferred anionic acid buffers include **citric** acid, succinic acid, phosphoric acid, maleic acid, and malonic acid.

DETD TABLE 5

Anionic Acid Buffers for Anodic Reservoir

BUFFER	ANIONIC pH RANGE	PREFERRED ANIONIC pH RANGE
citric acid	3-8	4-7
succinic acid	3-7.5	4-6.5
phosphoric acid	3-9	4-8
maleic acid	3-7.5	4-7
malonic acid	3-7.5	4-7
acetic acid	3-6	4-5.5
boric acid	8-10.5. . .	

DETD Alternatively, the anodic reservoir may be buffered using a

buffer which has a relatively high anodic electrotransport rate, ie, a cationic or positively charged **buffer** which buffers the anodic reservoir at a pH greater than about 4, preferably at a pH of about 4 to. . . In addition, mixtures of an acid from Table 5 and a base from Table 6 may also be used to **buffer** the anodic reservoir. However, the buffers of Table 5 are preferred over the buffers of Table 6, particularly when the. . . the agent for delivery into the body. The preferred bases for use in the anodic reservoir include tromethamine, triethanolamine and **imidazole**.

DETD TABLE 6

Cationic Bases and Amino Acids for the Anodic Reservoir

BASE **BUFFER**	pH RANGE FOR CATIONIC BEHAVIOR	PREFERRED pH RANGE FOR CATIONIC BEHAVIOR
tromethamine	6.8-9.3	7.3-8.8
triethanolamine	6.5-9	7-8.5
imidazole	5.8-8.2	6.3-7.7
ammonia	8-10.5	8.5-10
ethanolamine	8.2-10.8	8.8-10.2
diethanolamine	7.6-10.2	8.2-9.6
histidine	3-7.5	4-7.5
lysine	7.7-9.7	8.2-9.7
arginine	7.8-10.8	8.3-10.8

DETD The concentration of **buffer** required in the anodic reservoir, as in the cathodic reservoir, will depend on the properties of the specific **buffer** selected. Generally, the **buffer** concentration in the anodic reservoir will range from about 0.01M to about 1.0M. Preferably, the **buffer** concentration will be about 0.01M to about 0.50M. More preferably, the **buffer** concentration will be about 0.01M to about 0.20M.

DETD In those cases where competition from **buffer** ions/counterions must be minimized or eliminated, the **buffer** added to the anodic or cathodic reservoir is preferably polymeric. Examples of polymeric buffers include, without limitation, those listed in. . .

DETD TABLE 7

POLYMERIC **BUFFER**	ANIONIC pH RANGE	CATIONIC pH RANGE
polyacrylic acid	3-8	
polymethacrylic acid	3-8	
poly(styrene maleic anhydride)	3-8	
methacrylate/divinyl benzene copolymers.sup.1	3-8	
poly(2-acrylamido-2-methylpropane sulfonate)	1-5	
copolymers of acrylic acid and long chain. . .	3-8	

DETD . . . angiotensin II antagonists, antidiuretic hormone agonists, antidiuretic hormone antagonists, bradykinin antagonists, CD4, ceredase,

CSF's, enkephalins, FAB fragments, IgE peptide suppressors, **IGF** -1, neuropeptide Y, neurotrophic factors, oligodeoxynucleotides and their analogues such as antisense RNA, antisense DNA and anti-gene nucleic acids, opiate peptides,. . .

DETD In this set of experiments, sodium phosphate **buffer** (ie, negatively charged phosphate **buffer**) was added to the cathodic reservoir and various positively charged buffers having chloride counter ions were added to the anodic. . .

DETD TABLE 9

BUFFER	ANODIC RESERVOIR pH	SKIN RESISTANCE (KOhms .multidot. cm.sup.2) (.alpha.)	SKIN IRRITATION
0.15 M calcium chloride dihydrate	4.88	48.3	5.1
0.15 M triethanolamine hydrochloride	5.28	33.9	1.7
0.15 M magnesium	5.3. . .		

DETD These experiments involved the use of histidine, lysine, and **arginine**, all positively charged buffers in the form of chloride salts, in the cathodic reservoir and **citric** acid, monobasic sodium phosphate, and boric acid, all negatively charged buffers in the form of sodium salts in the anodic. . .

DETD . . . 10 for the cathodic reservoirs buffered with L-lysine or L-histidine; (ii) in Table 11 for the anodic reservoir buffered with **citric** acid, boric acid, or monobasic sodium phosphate, (iii) in Table 12 for the cathodic reservoirs buffered with L-histidine, L-lysine, or L-**arginine**, and (iv) in Table 13 for the cathodic reservoirs buffered with phosphoric acid and monobasic sodium phosphate.

DETD . . . 3%

HPC, NaOH q.s. to desired pH, and water q.s.)	ANODIC RESERVOIR (R) pH	SKIN RESISTANCE (KOhms .multidot. cm.sup.2) (.alpha.)	SKIN IRRITATION
0.05 M **citric** acid	2.11	51.6	4.7
0.05 M **citric** acid	2.72	50.9	5.3
0.05 M **citric** acid	3.62	45.6	4.0
0.05 M **citric** acid	4.52	33.2	2.7
0.05 M **citric** acid	5.31	29.6	2.7
0.05 M **citric** acid	6.55	27.4	3.3
0.05 M sodium phosphate monobasic	7.47	23.4	2.6
0.05 M boric acid	8.80	22.5	2.5

. . 9.93. . .
DETD . . . L-histidine base
6.00 7.0
0.05 M L-lysine base
6.55 6.4
0.05 M L-histidine base
7.09 6.6
0.05 M L-lysine base
8.98 6.4
0.05 M L-**arginine** base
10.19 6.4

DETD Because the **buffer** ions in this Example had a charge which was opposite the charge on the electrode (ie, positively charged **buffer** ions in the cathodic reservoir and negatively charged **buffer** ions in the anodic reservoir), a negligible amount of **buffer** ions were transported into the skin by electromi-gration. The skin irritation and skin resistance results were similar to those obtained. . . Furthermore, buffering of the reservoirs alone is sufficient to reduce skin irritation and skin resistance, and the charge of the **buffer** ion (which charge effects whether or not the **buffer** ion is delivered at a significant rate into the skin) does not appear to significantly affect the results, at least. . .

DETD Cathodic reservoir compositions containing sodium phosphate buffers (negatively charged **buffer** ions) were evaluated in this set of experi-ments. All compositions contained 0.05M phosphoric acid, 0.1M sodium chloride, 6% polyvinyl alcohol,. . .

DETD In this example, negatively charged phosphate **buffer** ions were present in the cathodic reservoir and hence, were transported into the skin. As FIGS. 11 and 12 illustrate,. . .

DETD Cathodic reservoir formulations containing histidine chloride **buffer** (ie, positively charged histidine **buffer** ions) and sodium citrate **buffer** (ie, negatively charged citrate **buffer** ions) at pH 3 and 4 were studied. All compositions contained 0.1M **buffer** (histidine or citrate), 6% polyvinyl alcohol (PVA), 4% hydroxypropylmethylcellulose (HPMC), either HCl (for histidine) or NaOH (for **citric** acid) q.s. to the desired pH, and q.s. water.

DETD . . . hours after the end of wearing. Both R and .alpha. values are given in Table 15 as a function of **buffer**, pH, and number of hours after removal. Each data point for pH 4 represents an average of readings for seven. . .

DETD . . . 13 and 14 show plots of skin irritation (.alpha.) and skin resistance (R), respectively, versus hours after device removal for **citric** acid and histidine buffers at pH 4. FIGS. 15 and 16 show plots of skin irritation (.alpha.) and skin resistance (R), respectively, versus hours after device removal for **citric** acid and histidine buffers at pH 3.

DETD In one set of tests, the cathodic reservoir contained positively charged histidine **buffer**, while in the other set, the cathodic reservoir contained negatively charged citrate **buffer**. Hence, the citrate **buffer** ions were transported into the skin by electromigration while the histidine **buffer** ions were not. Although skin resistance values for the two buffers were not significantly different (see FIGS. 14 and 16), buffering the cathodic reservoir with histidine resulted in lower skin irritation compared to buffering with **citric** acid (see FIGS. 13 and 15). Therefore, preventing or at least minimizing **buffer** transport into the skin (ie, through use of a cationic **buffer** in the cathodic reservoir and/or an anionic **buffer** in the anodic reservoir) is preferred for minimizing skin irritation.

DETD Cathodic reservoir formulations containing histidine chloride **buffer** adjusted to the desired pH with hydrochloric acid were placed on chest and arm sites of five different groups of. . .

DETD . . . . as a model drug salt). The cathodic reservoirs were buffered to

pH 3 or 4 using histidine, a positively charged **buffer** in the form of the chloride salt, and the anodic reservoirs were buffered to pH

5, 6 and 7 using **citric** acid, a negatively charged **buffer** in the form of its sodium salt. All reservoir compositions contained about 3% by weight hydroxyethylcellulose (HEC) and q.s. water.. . .

CLM What is claimed is:

4. The method of claim 3, wherein said anodic reservoir is buffered with

an anionic **buffer**.

5. The method of claim 3, wherein the anodic reservoir is buffered with a **buffer** selected from the group consisting of **citric** acid and EDTA.

9. The method of claim 8, wherein said cathodic reservoir is buffered with a cationic **buffer**.

10. The method of claim 9, wherein said cationic reservoir is buffered, during electrotransport agent delivery, with a cationic **buffer** selected from the group consisting of histidine, lysine, **arginine**, aspartic acid, glutamic acid, cysteine, tyrosine, and combination thereof.

11. The method of claim 8, wherein said cathodic reservoir is buffered with: (a) a polymeric **buffer** selected from the group consisting of vinylpyrrolidone/quaternized dimethylamino-ethylmethacrylate copolymers, vinylcaprolactam/vinylpyrrolidone/dimethyl amino ethylmethacrylate terpolymers, polyvinylpyrrolidone, and methacrylate/divinyl benzene copolymers; or (b) a **buffer** selected from the group consisting of aspartic acid, glutamic acid, **citric** acid, succinic acid, phosphoric acid, acetic acid, EDTA, lactic acid, benzoic acid, tartaric acid, maleic acid, fumaric acid, sulfuric acid,. . .

12. The method of claim 8 wherein the cathodic reservoir is buffered with a **buffer** selected from the group consisting of **citric** acid and EDTA.

. . . electrotransport agent delivery, the anodic reservoir at a pH above 4, wherein said anionic reservoir is buffered with an anionic **buffer** selected from the group consisting of histidine, lysine, **arginine**, aspartic acid, glutamic acid, cysteine, tyrosine, and combinations thereof; and (b) maintaining, during electrotransport agent

delivery, the cathodic reservoir t. . .

. . . during electrotransport agent delivery, the anodic reservoir at a pH above 4, wherein said anodic reservoir is buffered with a **buffer** selected from the group consisting of: (i) aspartic acid, glutamic acid,

succinic acid, phosphoric acid, acetic acid, lactic acid, boric. . . maleic acid, fumaric acid, sulfuric acid, formic acid, malic acid, malonic acid, glutaric acid, and adipic acid; (ii) tromethamine, triethanolamine, **imidazole**, ammonia, ethanolamine, diethanolamine, histidine, lysine, and **arginine**; (iii) calcium chloride dihydrate, triethanolamine hydrochloride, magnesium chloride heptahydrate, diethanolamine hydrochloride, ammonium chloride, ethanolamine hydrochloride, potassium chloride, and sodium chloride;.

L15 ANSWER 10 OF 13 USPATFULL

SUMM . . . for example Jacobsen et al U.S. Pat. No. 4,416,274 (sodium

. . phosphate buffers) and Hillman et al U.S. Pat. No. 5,088,978 (**citric** acid/citrate salt buffers). Although conventional buffers are effective to maintain donor reservoir pH, they introduce undesirable extraneous ions which tend. . . anodic donor reservoir for delivering a cationic drug D.sup.+ is buffered with a citrate salt (eg, sodium citrate), the citrate **buffer** absorbs hydronium ions produced by water hydrolysis at the anode but leaves extraneous sodium ions which compete with the drug. . .

SUMM . . . blistering of the skin in contact with either the cathode or anode is dependent upon both the pH and the **buffer** composition of the anodic and cathodic reservoirs.

SUMM . . . Del. Rev. (1992), 9, 239-264, the preferred pH range for avoiding skin irritation for the donor reservoir, independent of the **buffer** used, is 3 to 8. Outside this pH range, according to this reference, irritation and/or damage of the stratum corneum. . .

SUMM In a further preferred embodiment, the pH of one or both of the reservoirs is maintained using a suitable **buffer**. Most preferably, the cathodic reservoir is buffered using a cationic **buffer** and/or the anodic reservoir is buffered using an anionic **buffer**. Most preferably, the cathodic and/or anodic electrodes are composed of electrochemically reactive materials, ie, an electrochemically oxidizable anode and an. . .

DETD . . . cathode) and/or for short periods of time (eg, <1 hour), it may be sufficient to simply add an acid (eg **citric** acid) to the cathodic reservoir to maintain the desired pH. However, while acids are effective in achieving a low cathodic. . . reservoir pH, they introduce undesirable competing ions in those instances where the cathodic electrode is the donor electrode. Thus, adding **citric** acid to a cathodic donor reservoir containing salicylate anions undesirable adds citrate ions which compete with the salicylate ions for. . .

DETD Preferably, the cathodic reservoir contains at least one cationic **buffer**. A **buffer** cation within the cathodic reservoir will tend not to be electrotransported through the skin since anions, and not cations, are predominantly delivered from the cathodic reservoir by electrotransport. A poorly transported **buffer** is preferred in order to avoid depletion of the **buffer** from the reservoir as well as any irritation associated with **buffer** ion being transported into the skin. Amino acids are preferred cationic buffers. Preferably, the counter ion, ie anion, to the **buffer** cation is chloride. In many cases, the counter anions to the **buffer** ions are transported into the skin from the cathodic reservoir. Chloride is a preferred counter anion because the skin has. . .

DETD The concentration of **buffer** required in the reservoir will depend on the properties of the specific **buffer** selected. Generally, the **buffer** concentration will range from about 0.01M to about 1.0M. Preferably, the **buffer** concentration will be about 0.01M to about 0.50M. More preferably, the **buffer** concentration will be about 0.01M to about 0.20M.

DETD . . . Acid Buffers for Cathodic Reservoir

AMINO ACID	pH RANGE FOR CATIONIC BEHAVIOR	PREFERRED pH RANGE FOR CATIONIC BEHAVIOR
histidine	1-5	2-4
lysine	1-4	1.5-3.5
arginine	1-4	1.5-3.5

aspartic acid	1-3	2-3
glutamic acid	1-3.2	2-3.2
cysteine	1-4	2-3
tyrosine	1-4	2-3
other amino acids	1-4	2-3.5

DETD Alternatively, the cathodic reservoir may be buffered using an anionic or negatively charged **buffer**, which is electrotransported through the skin, or alternatively, mixtures of a cationic **buffer** from Table 1 and an anionic **buffer** from Table 2 may also be used. However, the cationic buffers of Table 1 are preferred, particularly when the cathodic electrode is the donor electrode, since **buffer** cations will not be electrotransported through the skin. Thus, irritation from the presence of a **buffer** ion in the skin is minimized, as discussed above. The preferred anionic buffers include those named in TABLE 2.

DETD . . . no buffering capacity may optionally be incorporated into the cathodic reservoir. Such additives may be advantageous in decreasing the potential **buffer** depletion from the reservoir. A disadvantage of adding sodium chloride to the reservoir, at least in the case where the. . .

DETD TABLE 2

Anionic Acid Buffers for Cathodic Reservoir

BUFFER	pH RANGE FOR ANIONIC BEHAVIOR	PREFERRED pH RANGE FOR ANIONIC BEHAVIOR
aspartic acid	3-5	3-4
glutamic acid	3.2-5	3.2-4
citric acid	1-5	2-4
succinic acid	2-5	3-4
phosphoric acid	1-5	2-4
acetic acid	3.5-5	3.5-4
EDTA	1-5	2-4
tactic acid	2.7-4.5	2.7-4
benzoic acid	3-5	3-4

tartaric. . .

DETD . . . (eg, platinum or stainless steel), the anodic reservoir is preferably buffered at a pH above about 4. More preferably, the **buffer** has a relatively low anodic electrotransport rate through the skin. Preferred buffers include amino acids exhibiting anionic behavior at a. . .

DETD . . . Reservoir

AMINO ACID	pH RANGE FOR ANIONIC BEHAVIOR	PREFERRED pH RANGE FOR ANIONIC BEHAVIOR
histidine	7.5-10.5	7.5-10
cysteine	7-12	7.5-11.5
tyrosine	7.8-11.4	8.3-10.9
lysine	9.7-11.8	9.7-11.3

arginine	10.8-13	10.8-12
aspartic acid		
	3-5.2	4-4.6
	8.5-11.1	9.1-10.5
glutamic acid		
	3-5.5	4-5
	8.4-11	8.9-10.4
other amino acids		
	8-12	9-11

DETD . . . for the anodic reservoir. Examples of such acids can be found in TABLE 5. The preferred anionic acid buffers include **citric** acid, succinic acid, phosphoric acid, maleic acid, and malonic acid.

DETD TABLE 5

Anionic Acid Buffers for Anodic Reservoir

BUFFER	ANIONIC pH RANGE	PREFERRED ANIONIC pH RANGE
citric acid	3-8	4-7
succinic acid	3-7.5	4-6.5
phosphoric acid	3-9	4-8
maleic acid	3-7.5	4-7
malonic acid	3-7.5	4-7
acetic acid	3-6	4-5.5
boric acid	8-10.5. . .	

DETD Alternatively, the anodic reservoir may be buffered using a **buffer** which has a relatively high anodic electrotransport rate, ie, a cationic or positively charged **buffer** which buffers the anodic reservoir at a pH greater than about 4, preferably at a pH of about 4 to. . . In addition, mixtures of an acid from Table 5 and a base from Table 6 may also be used to **buffer** the anodic reservoir. However, the buffers of Table 5 are preferred over the buffers of Table 6, particularly when the. . . the agent for delivery into the body. The preferred bases for use in the anodic reservoir include tromethamine, triethanolamine and **imidazole**.

DETD TABLE 6

Cationic Amino Acids for the Anodic Reservoir

BASE **BUFFER** CATIONIC BEHAVIOR	pH RANGE FOR PREFERRED CATIONIC BEHAVIOR	pH RANGE FOR PREFERRED CATIONIC BEHAVIOR
tromethamine	6.8-9.3	7.3-8.8
triethanolamine	6.5-9	7-8.5
imidazole	5.8-8.2	6.3-7.7
ammonia	8-10.5	8.5-10
ethanolamine	8.2-10.8	8.8-10.2
diethanolamine	7.6-10.2	8.2-9.6
histidine	3-7.5	4-7.5
lysine	7.7-9.7	8.2-9.7
arginine	7.8-10.8	8.3-10.8

DETD The concentration of **buffer** required in the anodic reservoir, as in the cathodic reservoir, will depend on the properties of the specific **buffer** selected. Generally, the **buffer** concentration in the anodic reservoir will range from about 0.01M to about 1.0M. Preferably, the **buffer** concentration will be about 0.01M to about 0.50M. More preferably, the **buffer** concentration will be about 0.01M to about 0.20M.

DETD In those cases where competition from **buffer** ions/counterions must be minimized or eliminated, the **buffer** added to the anodic or cathodic reservoir is preferably polymeric. Examples of polymeric buffers include, without limitation, those listed in. . .

DETD TABLE 7

POLYMERIC **BUFFER**	ANIONIC pH RANGE	CATIONIC pH RANGE
polyacrylic acid	3-8	
polymethacrylic acid	3-8	
poly(styrene maleic anhydride)	3-8	
methacrylate/divinyl benzene copolymers.sup.1	3-8	
poly(2-acrylamido-2-methylpropane sulfonate)	1-5	
copolymers of acrylic acid and long chain acrylate esters.sup.2	3-8	
poly(methylvinyl. . .		

DETD . . . angiotensin II antagonists, antidiuretic hormone agonists, antidiuretic hormone antagonists, bradykinin antagonists, CD4, ceredase, CSF's, enkephalins, FAB fragments, IgE peptide suppressors, **IGF**-1, neuropeptide Y, neurotrophic factors, oligodeoxynucleotides and their analogues such as antisense RNA, antisense DNA and anti-gene nucleic acids, opiate peptides,. . .

DETD In this set of experiments, sodium phosphate **buffer** (ie, negatively charged phosphate **buffer**) was added to the cathodic reservoir and various positively charged buffers having chloride counter ions were added to the anodic. . .

DETD TABLE 9

BUFFER	ANODIC RESERVOIR pH	SKIN RESISTANCE (KOhms .multidot. cm.sup.2) (.alpha.)	SKIN IRRITATION
0.15M calcium chloride dehydrate	4.88	48.3	5.1
0.15M triethanolamine hydrochloride	5.28	33.9	1.7
0.15M magnesium chloride heptahydrate	5.3	41.7	2.6
0.15M diethanolamine			

DETD These experiments involved the use of histidine, lysine, and **arginine**, all positively charged buffers in the form of chloride salts, in the cathodic reservoir and **citric** acid, monobasic sodium phosphate, and boric acid, all negatively charged buffers in the form of sodium salts in the anodic. . .

DETD . . . 10 for the cathodic reservoirs buffered with L-lysine or L-histidine; (ii) in TABLE 11 for the anodic reservoir buffered with **citric** acid, boric acid, or monobasic sodium phosphate, (iii) in TABLE 12 for the cathodic reservoirs buffered with L-histidine, L-lysine, or L-**arginine**, and (iv) in TABLE 13 for the cathodic reservoirs buffered with phosphoric acid and monobasic sodium phosphate.

DETD . . . NaCl, 3% HPC, NaOH q.s. to desired pH, and water q.s.)

RESERVOIR pH	ANODIC RESISTANCE (R) (KOhms .multidot. cm.sup.2) (.alpha.)	SKIN IRRITATION
0.05M **citric** acid 2.11	51.6	4.7
0.05M **citric** acid 2.72	50.9	5.3
0.05M **citric** acid 3.62	45.6	4.0
0.05M **citric** acid 4.52	33.2	2.7
0.05M **citric** acid 5.31	29.6	2.7
0.05M **citric** acid 6.55	27.4	3.3
0.05M sodium phosphate monobasic 7.47	23.4	2.6
0.05M boric acid 8.80	22.5	2.5
0.05M boric acid 9.93	23.5	2.5

DETD . . . L-histidine base

0.05M L-histidine base	4.93	7.1
0.05M L-lysine base	6.00	7.0
0.05M L-histidine base	6.55	6.4
0.05M L-lysine base	7.09	6.6
0.05M L-**arginine** base	8.98	6.4
	10.19	6.4

DETD Because the **buffer** ions in this Example had a charge which was opposite the charge on the electrode (ie, positively charged **buffer** ions in the cathodic reservoir and negatively charged **buffer** ions in the anodic reservoir), a negligible amount of **buffer** ions were transported into the skin by electromigration. The skin irritation and skin resistance results were similar to those obtained. . . Furthermore, buffering of the reservoirs alone is sufficient to reduce skin irritation and skin resistance, and the charge of the **buffer** ion (which charge effects whether or not the **buffer** ion is delivered at a significant rate into the skin) does not appear to significantly affect the results, at least. . .

DETD Cathodic reservoir compositions containing sodium phosphate buffers

(negatively charged **buffer** ions) were evaluated in this set of experiments. All compositions contained 0.05M phosphoric acid, 0.1M sodium chloride, 6% polyvinyl alcohol,. . .

DETD In this EXAMPLE, negatively charged phosphate **buffer** ions were present in the cathodic reservoir and hence, were transported into the skin. As FIGS. 11 and 12 illustrate,. . .

DETD Cathodic reservoir formulations containing histidine chloride **buffer** (ie, positively charged histidine **buffer** ions) and sodium citrate **buffer** (ie, negatively charged citrate **buffer** ions) at pH 3 and 4 were studied. All compositions contained 0.1M **buffer** (histidine or citrate), 6% polyvinyl alcohol (PVA), 4% hydroxypropylmethylcellulose (HPMC), either HCl (for histidine) or NaOH (for **citric** acid) q.s. to the desired pH, and q.s. water.

DETD . . . hours after the end of wearing. Both R and .alpha. values are given in TABLE 15 as a function of **buffer**, pH, and number of hours after removal. Each data point for pH 4 represents an average of readings for seven. . .

DETD . . . 13 and 14 show plots of skin irritation (.alpha.) and skin resistance (R), respectively, versus hours after device removal for **citric** acid and histidine buffers at pH 4. FIGS. 15 and 16 show plots of skin irritation (.alpha.) and skin resistance (R), respectively, versus hours after device removal for **citric** acid and histidine buffers at pH 3.

DETD In one set of tests, the cathodic reservoir contained positively charged histidine **buffer**, while in the other set, the is cathodic reservoir contained negatively charged citrate **buffer**. Hence, the citrate **buffer** ions were transported into the skin by electromigration while the histidine **buffer** ions were not. Although skin resistance values for the two buffers were not significantly different (See FIGS. 14 and 16), buffering the cathodic reservoir with histidine resulted in lower skin irritation compared to buffering with **citric** acid (See FIGS. 13 and 15). Therefore, preventing or at least minimizing **buffer** transport into the skin (ie, through use of a cationic **buffer** in the cathodic reservoir and/or an anionic **buffer** in the anodic reservoir) is preferred for minimizing skin irritation.

DETD Cathodic reservoir formulations containing histidine chloride **buffer** adjusted to the desired pH with hydrochloric acid were placed on chest and arm sites of five different groups of. . .

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DETD . . . practice of the invention mentioned above include nerve growth factors (NGF, NGF-2/NT-3), epidermal growth factor (EGF), fibroblast growth factor (FGF), **insulin-like growth factor** (**IGF**), transforming growth factor (TGF), platelet-derived cell growth factor (PDGF), hepatocyte growth factor (HGF) and so on.

DETD . . . base necessary for dispersion of such substances. Said additives include but are not limited to pH control agents, such as **arginine**, sodium hydroxide, glycine, hydrochloric acid, **citric** acid, etc., local anesthetics represented by benzyl alcohol, isotonizing agents such as sodium chloride, mannitol, sorbitol, etc., adsorption inhibitors such. . .

DETD . . . sodium lauryl sulfate, sodium myristyl sulfate, polyoxyethylene alkyl ethers, polyoxyethylene alkyl esters, etc.), caproic acid, lactic acid, malic acid and **citric** acid and alkali metal salts thereof, pyrrolidonecarboxylic acids, alkylpyrrolidonecarboxylic acid esters, N-alkylpyrrolidones, proline acyl esters and so on. While the. . .

DETD . . . form of p-bromobenzyloxycarbonyl ester, the carboxyl group of glutamic acid or aspartic acid in the form of benzyl ester, the **imidazole** nitrogen of hystidine with benzyloxy methyl, the

side-chain amino group of lysine with 2-chlorobenzyloxycarbonyl, the guanidino group of **arginine** with p-toluenesulfonyl, and the indoleimine group of tryptophan with formyl. All the amino acids were obtained from Applied Biosystems Japan. . .

DETD In 155.5 .mu.l of 1/10M **citric** acid **buffer** (pH 3.5) was suspended 16.75 mg of human insulin (Wako Pure Chemical Industries).

A 20 .mu.l of this suspension was. . .

DETD In 155.5 .mu.l of 1/10M citrate **buffer** (pH 3.5) was dispersed 16.75 mg of human insulin (Wako Pure Chemical) and 50 .mu.l of this dispersion was blended. . .

L15 ANSWER 12 OF 13 USPATFULL

SUMM Various ways are known to release growth hormone. For example, chemicals such as **arginine**, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth. . .

SUMM . . . 89/07111 and B-HT920 or growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins including **IGF**-1 and **IGF**-2.

DETD . . . 3 hours at room temperature, the mixture was diluted into 30 mL of ethyl acetate and washed with 5% aqueous **citric** acid, saturated aqueous sodium bicarbonate (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents. . .

DETD . . . After 1 hour at room temperature, the mixture was added to 20 mL ethyl acetate and washed with 1M aqueous **citric** acid, saturated aqueous sodium bicarbonate and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents removed.

.

DETD . . . temperature for 4 hours. The mixture was added to 30 mL ethyl acetate and washed twice with pH 7.0 phosphate **buffer** and once with brine. The organic layer was removed, dried over magnesium sulfate filtered and solvents removed in vacuo. The. . .

DETD . . . for 2 hours. The reaction mixture was added to 50 mL of ethyl acetate and washed with pH 7.0 phosphate **buffer** (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents removed under vacuum. Purification by. . .

DETD . . . mixture was stirred at room temperature for 20 hours then added to 100 mL ethyl acetate and washed with 5% **citric** acid (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvent removed under vacuum to. . .

DETD . . . After 2 hours at room temperature, the mixture was added to 30 mL of ethyl acetate and washed with 5% **citric** acid (2.times.), saturated aqueous sodium bicarbonate and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents. . .

DETD . . . 1 hour. The reaction mixture was added to 30 mL of ethyl acetate/hexane (1:1) and washed with pH 7.0 phosphate **buffer** and once with brine. The organic layer was removed, dried over magnesium sulfate filtered and solvents removed under vacuum. The. . .

DETD . . . for 48 hours then concentrated under vacuum. The residue was redissolved in ethyl acetate and the solution washed with 5% **citric** acid and brine, then dried over magnesium sulfate, filtered and evaporated under vacuum to afford 1.75 g (7.63 mmol, 98%). . .

DETD . . . two hours at room temperature, the mixture was added to 20 mL of ethyl acetate and washed with 5% aqueous **citric** acid, saturated aqueous sodium bicarbonate and brine. The organic layer was separated, dried over magnesium sulfate, filtered and solvents removed.

. . ..
DETD . . . After 2 hours the reaction was diluted with 75 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium. . .

DETD . . . After 2 hours, the reaction was diluted with 75 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . (6.2 mmol) of 2,3-isopropylidene-D-threitol in 6.0 mL of dry dimethylformamide at 0.degree. C. was added 0.44 g (6.5 mmol) of **imidazole** followed by dropwise addition of a solution of 0.93 g (6.2 mmol) of t-butyldimethylsilyl chloride in 6.0 mL of dimethylformamide.. . .

DETD . . . room temperature for 2 hours then diluted with 100 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . for 2 hours. The reaction was diluted with 75 mL of ethyl acetate, washed with 25 Ml of 5% aqueous **citric** acid, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium. . .

DETD . . . 1.5 g of 2-bromo-4-iodotoluene and heated briefly to 40.degree.

C. The mixture was cooled and partitioned between ether and saturated **citric** acid. The organic layer was separated, washed with brine (2.times.), dried over magnesium sulfate, filtered and concentrated under vacuum. The. . .

CLM What is claimed is:

. . . with other growth hormone secretagogues such as, GHRP-6 or GHRP-1, growth hormone releasing factor (GRF) or one of its analogs, **IGF**-1 or **IGF**-2, or B-HT920.

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SUMM Various ways are known to release growth hormone. For example, chemicals such as **arginine**, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth. . .

SUMM . . . 89/07111 and B-HT920 or growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins including **IGF**-1 and **IGF**-2.

DETD . . . 3 hours at room temperature, the mixture was diluted into 30 mL of ethyl acetate and washed with 5% aqueous **citric** acid, saturated aqueous sodium bicarbonate (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents. . .

DETD . . . After 1 hour at room temperature, the mixture was added to 20 mL ethyl acetate and washed with 1M aqueous **citric** acid, saturated aqueous sodium bicarbonate and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents removed.

.

DETD . . . temperature for 4 hours. The mixture was added to 30 mL ethyl acetate and washed twice with pH 7.0 phosphate **buffer** and once with brine. The organic layer was removed, dried over magnesium sulfate filtered and solvents removed in vacuo. The. . .

DETD . . . for 2 hours. The reaction mixture was added to 50 mL of ethyl acetate and washed with pH 7.0 phosphate **buffer** (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents removed under vacuum. Purification by. . .

DETD . . . mixture was stirred at room temperature for 20 hours then added

to 100 mL ethyl acetate and washed with 5% **citric** acid (2.times.) and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvent removed under vacuum to. . .

DETD . . . After 2 hours at room temperature, the mixture was added to 30 mL of ethyl acetate and washed with 5% **citric** acid (2.times.), saturated aqueous sodium bicarbonate and brine. The organic layer was removed, dried over magnesium sulfate, filtered and solvents. . .

DETD . . . 1 hour. The reaction mixture was added to 30 mL of ethyl acetate/hexane (1:1) and washed with pH 7.0 phosphate **buffer** and once with brine. The organic layer was removed, dried over magnesium sulfate filtered and solvents removed under vacuum. The. . .

DETD . . . for 48 hours then concentrated under vacuum. The residue was redissolved in ethyl acetate and the solution washed with 5% **citric** acid and brine, then dried over magnesium sulfate, filtered and evaporated under vacuum to afford 1.75 g (7.63 mmol, 98%). . .

DETD . . . two hours at room temperature, the mixture was added to 20 mL of ethyl acetate and washed with 5% aqueous **citric** acid, saturated aqueous sodium bicarbonate and brine. The organic layer was separated, dried over magnesium sulfate, filtered and solvents removed. . .

DETD . . . After 2 hours the reaction was diluted with 75 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium. . . .

DETD . . . After 2 hours, the reaction was diluted with 75 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . (6.2 mmol) of 2,3-isopropylidene-D-threitol in 6.0 mL of dry dimethylformamide at 0.degree. C. was added 0.44 g (6.5 mmol) of **imidazole** followed by dropwise addition of a solution of 0.93 g (6.2 mmol) of t-butyldimethylsilyl chloride in 6.0 mL of dimethylformamide.. . .

DETD . . . room temperature for 2 hours then diluted with 100 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium sulfate,. . .

DETD . . . for 2 hours. The reaction was diluted with 75 mL of ethyl acetate, washed with 25 mL of 5% aqueous **citric** acid, 25 mL of saturated aqueous sodium bicarbonate and 25 mL of brine. The organic layer was dried over magnesium. . .

DETD . . . 1.5 g of 2-bromo-4-iodotoluene and heated briefly to 40.degree. C. The mixture was cooled and partitioned between ether and saturated **citric** acid. The organic layer was separated, washed with brine (2.times.), dried over magnesium sulfate, filtered and concentrated under vacuum. The. . .